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Supplemental Material to:

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Prostate cancer stem cells are targets of both innate and adaptive immunity and elicit tumor-specific immune responses

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LEGENDS FOR SUPPLEMENTARY FIGURES

Supplementary Figure 1. PAC-SC and PNE-SC express prostate specific antigens A) Total RNA from the indicated samples was retrotranscribed to cDNA and submitted to 40 cycles of PCR amplification with STEAP, PSCA, Tag, PAP, BCRP or β-actin primers, respectively. All the PCR products were visualized on a 1.5% or 2.5% agarose gel stained with Sybr-safe. TRAMP-C1 cells and TRAMP prostatic tissue were used as positive controls (+). B) Cells were cultured on a round glass slide for 48h, fixed with PFA and tested for STEAP (red), PSCA (green) ot TAG (green) expression by ICC.

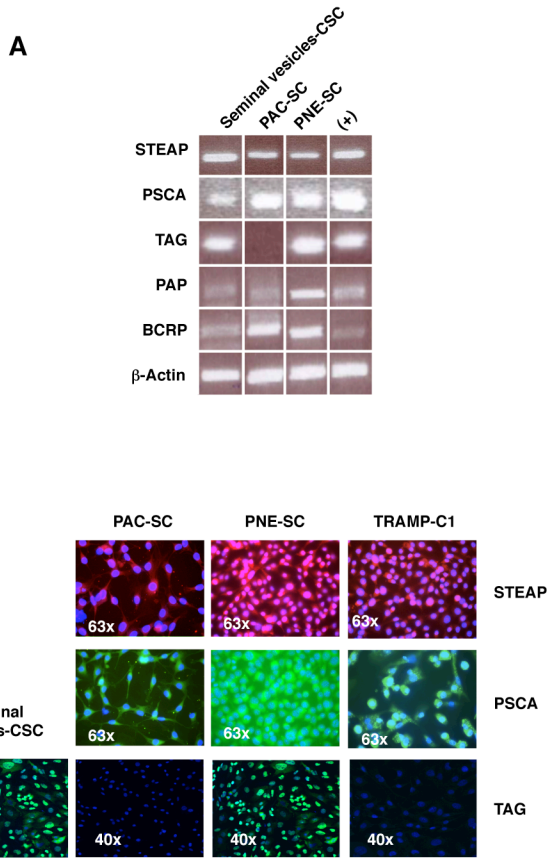
Supplementary Figure 2. PAC-SC and PNE-SC undergo apoptosis after γ-irradiation PAC-SC or PNE-SC were irradiated at 50 Gy and tested after 6h by flow cytometry for early (annexin-V^+ / $7AAD^-$) and late (annexin-V^+ / $7AAD^+$) apoptosis with the BD-Biosciences Apoptosis Kit.

Supplementary Figure 3. DC+PAC-SC immunization elicits a tumor-specific immune response in TRAMP mice. A) Upper panels; six-week old TRAMP mice were immunized with DC+PAC-SC or DC w/o ($5x10^5$ DC/mouse) and killed at 16 weeks of age. Alternatively (lower panels), 16-week old TRAMP mice were immunized with DC+PAC-SC and killed one week later. Their splenocytes were restimulated *in vitro* and tested 5 days later for intracellular IFNγ production (targets: RMA, left panels; PAC-SC, middle panels; TRAMP-C1, right panels). Dot plots are gated on $CD8^+$ T cells; the percentage of double positive CD44/IFNγ cells is indicated. B) Histograms show the quantification of IFNγ production against PAC-SC

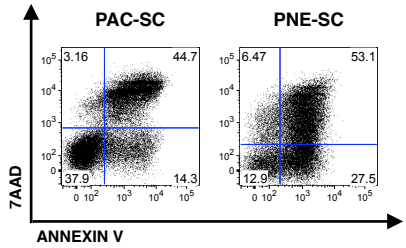
(left panel) or TRAMP-C1 (right panel). Values are subtracted of background (i.e. IFNγ production against the irrelevant target RMA). Each panel is representative of two independent experiments. Student's T test: *$p < 0.05$; ** $p < 0.01$.

Supplementary Figure 4. DC+STEAP immunization elicits a specific immune response. WT mice were immunized with DC+STEAP or DC w/o ($5x10^5$ DC/mouse) and killed after one week. Their splenocytes were then restimulated *in vitro* with $STEAP_{186-193}$ peptide and tested 5 days later for intracellular IFNγ production (targets: RMA, left panels; RMA pulsed with $STEAP_{186-193}$, right panels). Dot plots are gated on $CD8^+$ T cells; the percentage of double positive CD44/IFNγ cells is indicated.

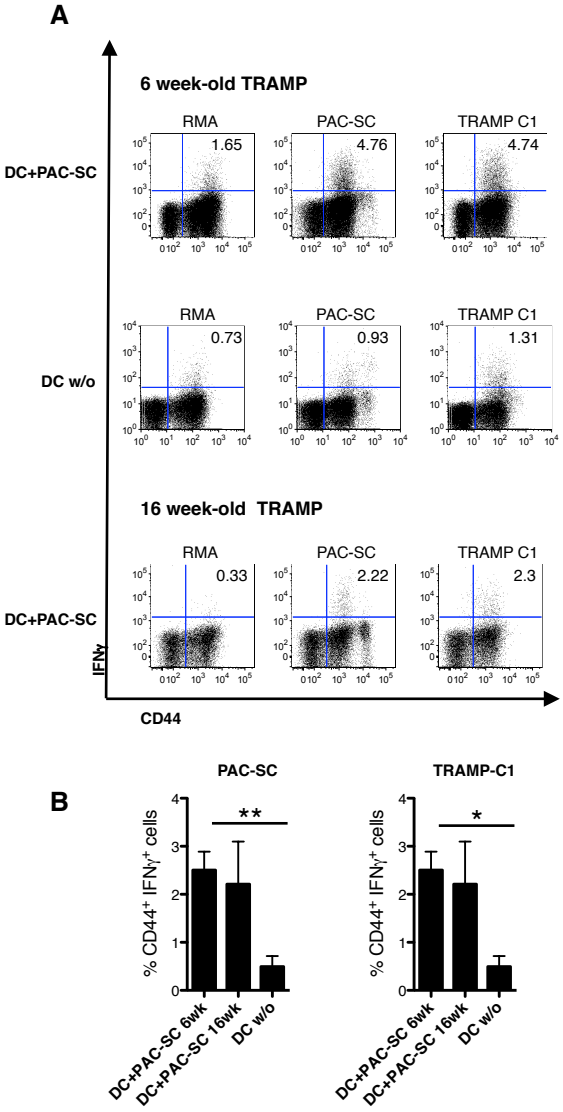
Jachetti E. et al. Suppl. Fig.1



Jachetti E. et al. Suppl. Fig.2



Jachetti E. et al. Suppl. Fig.3



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